

I Claim:

1. A method for recovering cryopreserved plant cells comprising the steps of:
  - a) thawing the cryopreserved plant cells to a temperature above freezing;
  - b) incubating the thawed plant cells in a growth medium containing an ethylene inhibitor; and
  - c) recovering viable plant cells.
2. The method of claim 1 wherein the plant cells are gymnosperm or angiosperm.
3. The method of claim 2 wherein the gymnosperm is a species of *Abies*, *Cypressus*, *Ginkgo*, *Juniperus*, *Picea*, *Pinus*, *Pseudotsuga*, *Sequoia*, *Taxus*, *Tsuga* or *Zamia*.
4. The method of claim 3 wherein the *Taxus* species is *T. baccata*, *T. brevifolia*, *T. canadensis*, *T. chinensis*, *T. cuspidata*, *T. floridana*, *T. globosa*, *T. media*, *T. nucifera* or *T. wallichiana*.
5. The method of claim 2 wherein the angiosperm comprises monocotyledon plant cells or dicotyledon plant cells.
6. The method of claim 5 wherein the monocotyledon plant cell is selected from the group consisting of species of the genus *Avena*, *Cocos*, *Dioscorea*, *Hordeum*, *Musa*, *Oryza*, *Saccharum*, *Sorghum*, *Triticum* and *Zea*.

7. The method of claim 5 wherein the dicotyledon plant cells are selected from the group consisting of species of the genus *Achyrocline*, *Atropa*, *Brassica*, *Berberis*, *Capsicum*, *Catharanthus*, *Conospermum*, *Datura*, *Daucus*, *Digitalis*, *Echinacea*, *Eschscholtzia*, *Glycine*,  
 5 *Gossypium*, *Hyoscyamus*, *Legumes*, *Lupinus*, *Lycopersicum*, *Malus*, *Medicago*, *Nicotiana*, *Panax*, *Pisum*, *Rauvolfia*, *Ruta*, *Solanum*, *Sophora* and *Trichosanthes*.
8. The method of claim 1 wherein the cryopreserved plant cells are thawed to about room temperature.
- 10 9. The method of claim 1 wherein the ethylene inhibitor is an ethylene biosynthesis inhibitor or an ethylene action inhibitor.
10. The method of claim 9 wherein the ethylene action inhibitor is a silver salt.
11. The method of claim 10 wherein the silver salt is selected from the  
 15 group consisting of silver thiosulfate, silver nitrate, silver chloride, silver acetate, silver phosphate, silver sulfate, silver nitrite and combinations thereof.
12. The method of claim 9 wherein the ethylene biosynthesis inhibitor is selected from the group consisting of spermidine, spermine, catechol,  
 20 n-propyl gallate, hydroquinone, ferulic acid, alar, phenylethylamine, salicyl alcohol, indomethacin and combinations thereof.

13. The method of claim 1 wherein <sup>the</sup> incubating and <sup>the</sup> recovering is performed in a liquid medium.

14. The method of claim 1 wherein <sup>the</sup> incubating is performed on a semi-solid medium.

15. The method of claim 1 wherein greater than about 50% of recovered plant cells are viable.

16. The method of claim 1 wherein the growth medium further comprises a divalent cation.

17. The method of claim 16 wherein the divalent cation is calcium, magnesium or manganese.

18. The method of claim 1 wherein the growth medium further comprises a cryoprotective agent.

19. The method of claim 18 wherein the cryoprotective agent is selected from the group consisting of sorbitol, mannitol, sucrose, trehalose, proline and mixtures thereof.

20. Viable plant cells recovered by the method of claim 1.

21. A plant propagated from viable plant cells of claim 21.

22. A method for recovering cryopreserved plant cells comprising the steps of:
- a) thawing the cryopreserved plant cells to a temperature above freezing;
  - 5 b) incubating the thawed plant cells in a growth medium containing a divalent cation; and
  - c) recovering viable plant cells.
23. The method of claim 22 wherein the divalent cation is calcium, magnesium or manganese.
- 10 24. The method of claim 22 wherein the growth medium further comprises a cryoprotective agent.
25. The method of claim 22 wherein the growth medium further comprises an ethylene inhibitor.
26. The method of claim 22 wherein the viable plant cells recovered  
15 have a vigorous regrowth.
27. Viable plant cells recovered by the method of claim 22.
28. A method for cryopreserving a plant cell comprising the steps of:
- a) pretreating the plant cell with an osmotic agent and a divalent cation at greater than about 5 mM;
  - 20 b) loading the plant cell with a cryopreserving agent;
  - c) vitrifying the plant cell with a cryopreservation solution; and

- d) freezing the vitrified plant cell at a cryopreservation temperature.

29. The plant cell of claim 28 which is a species of *Taxus*, *Solanum*, *Legume*, *Lycopersicum* or *Nicotiana*.

- 5 30. The method of claim 28 wherein the plant cell is obtained from new growth needles, bark, leaves, stem, root, rhizome, callus cells, protoplasts, cell suspensions, meristems, seeds or embryos.

31. The method of claim 28 wherein pretreatment involves culturing said plant cell in medium containing the osmotic agent and the divalent  
10 cation for between about 1 day and about 6 days.

32. The method of claim 28 wherein the osmotic agent is sucrose, sorbitol or mannitol at a concentration of between about 0.06 M and about 0.8 M.

33. The method of claim 28 wherein the divalent cation is  $\text{CaCl}_2$ ,  
15  $\text{MgCl}_2$  or  $\text{MnCl}_2$ .

34. The method of claim 28 wherein the divalent cation is at a concentration of from about 5 mM to about 20 mM.

35. The method of claim 28 wherein loading comprises incubating said plant cell in the cryoprotection solution comprising between about 0.5%  
20 to about 30%, by weight, of the cryoprotecting agent.

36. The method of claim 35 wherein the cryoprotecting agent is selected from the group consisting of DMSO, propylene glycol, glycerol, polyethylene glycol, ethylene glycol, butanediol, formamide, propanediol, sorbitol, mannitol and mixtures thereof.
- 5 37. The method of claim 28 wherein the cryoprotecting agent is selected from the group consisting of DMSO, propylene glycol, glycerol, polyethylene glycol, ethylene glycol, butanediol, formamide, propanediol, sorbitol, mannitol and mixtures thereof.
38. The method of claim 28 wherein the osmotic agent and the  
10 cryoprotecting agent are the same.
39. The method of claim 28 wherein loading and vitrifying are conducted simultaneously.
40. The method of claim 28 wherein loading or vitrifying is performed in a single step or in a plurality of steps.
- 15 41. The method of claim 40 wherein the plurality of steps comprises adding the cryoprotecting agent to the plant cell five times at one minute intervals.
42. The method of claim 28 wherein the cryopreservative temperature is less than about  $-70^{\circ}\text{C}$ .

43. The method of claim 28 further comprising the step of including a stabilizer during pretreatment, loading or vitrification.
44. The method of claim 43 wherein the stabilizer is a divalent cation, an oxygen radical scavenger, an ethylene inhibitor or a heat-shock protein.
- 5 45. The method of claim 44 wherein the ethylene inhibitor is an ethylene biosynthesis inhibitor or an ethylene action inhibitor.
46. The method of claim 28 further comprising the step of storing the cryopreserved cell at said cryopreservation temperature for a period of time of greater than one month.
- 10 47. The method of claim 46 wherein the period of time is greater than one year.
48. A viable plant cell cryopreserved by the method of claim 28.
49. The viable plant cell of claim 48 wherein said cell is not significantly phenotypically or genetically altered by cryopreservation.
- 15 50. A method for recovering cryopreserved plant cells comprising the steps of:
- a) cryopreserving plant cells according to the method of claim 28;
  - b) thawing the cryopreserved plant cells to a temperature above
- 20 freezing;

- c) incubating the thawed plant cells in a growth medium containing a stabilizer;
  - d) removing the cryoprotective agent; and
  - e) recovering viable plant cells.
- 5 51. The method of claim 50 wherein the stabilizer is a divalent cation, an oxygen radical scavenger, an ethylene inhibitor or a combination thereof.
52. The method of claim 50 wherein the growth medium further contains a cyroprotectant.
- 10 53. A method for cryopreserving a plant cell comprising the steps of:
- a) pretreating the plant cell with an osmotic agent and an ethylene inhibitor;
  - b) loading the plant cell with a cryopreserving agent;
  - c) vitrifying the plant cell with a cryopreservation solution; and
  - 15 d) freezing the vitrified plant cell at a cryopreservation temperature.
54. The method of claim 53 wherein the ethylene inhibitor is an ethylene biosynthesis inhibitor or an ethylene action inhibitor.
55. The method of claim 53 further comprising the step of adding
- 20 divalent cations to the pretreating, loading or vitrifying steps.
56. A method for cryopreserving a plant cell comprising the steps of:



- a) pretreating the plant cell with a heat shock;
- b) vitrifying the acclimated plant cell with a vitrification solution; and
- c) freezing the incubated plant cell at a cryopreservation temperature.

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57. The method of claim 56 wherein the plant cell is a species of *Taxus*, *Solanum*, *Legume*, *Lycopersicum* or *Nicotiana*.

58. The method of claim 56 wherein the heat shock comprises incubation of the plant cell at about 37°C from about 2 to about 4 hours.

10 59. The method of claim 56 further comprising the step of including a divalent cation during pretreating, vitrifying or both pretreating and vitrifying.

60. A viable plant cell cryopreserved by the method of claim 56.